

# Clinical relevance of dual agonist of glucagon and glucagon-like peptide-1 receptors to achieve functional restoration of first- and second-phase insulin secretion

Glucose-stimulated insulin secretion (GSIS) from pancreatic  $\beta$ -cells plays an important role not only to maintain systemic glucose homeostasis in insulin target tissues, but also to predispose pancreatic  $\beta$ -cell dysfunction or to give a diagnosis of diabetes. It is well-known that there is a cross association of GSIS with the increment of intracellular  $\text{Ca}^{2+}$ , mainly produced by glucose uptake and oxidative glucose metabolism. Furthermore, the kinetic study of GSIS in response to a stepwise increase of glucose concentration, as used in the islet perfusion study, showed the two distinct components defined as the first- and second-phase insulin secretion. In the first phase, but not the second phase, GSIS in response to glucose alone was abolished by the addition of Rp-8-Br-cAMPS-pAB, a cyclic adenosine monophosphate (cAMP) antagonist prodrug<sup>1</sup>, strongly suggesting that cAMP is an essential cofactor of the first-phase GSIS.

Thereby, it is hypothesized that the first-phase GSIS might be regulated by intra-islet paracrine hormones, such as glucagon and glucagon-like peptide-1 (GLP-1). Actually, a line of evidence has shown that glucagon from  $\alpha$ -cells stimulated cAMP production, which is involved in GSIS as a necessary cofactor, through binding to  $\beta$ -cell glucagon receptors (GcgR) and/or GLP-1 receptors

(GLP-1R)<sup>2-4</sup>. Svendsen *et al.*<sup>4</sup> reported that complete blockade of glucagon signaling in islets severely limits insulin secretion, and showed that paracrine glucagon acts on both glucagon and GLP-1 receptors. They concluded that combined activity of GcgR and GLP-1R is necessary to the secretory responses of  $\beta$ -cells, accentuating a role for paracrine intra-islet glucagon actions to maintain appropriate insulin secretion. Thus, it is obvious that paracrine glucagon action through both GcgR and GLP-1R is definitely essential to maintain normal insulin secretion.

Recently, Cabrera *et al.*<sup>5</sup> integrated understanding of GSIS by examining in more detail the effects of endogenous glucagon, extrinsic glucagon,  $\alpha$ -cells to  $\beta$ -cells communication, various receptors expressed on  $\beta$ -cells and cAMP production involved in insulin secretion. They showed GSIS in the following three conditions using rat islets. First, glucagon secreted from  $\alpha$ -cells activates GcgR in  $\beta$ -cells and promotes GSIS when glucose alone is loaded. In this condition, GSIS is blocked by LY2786890, a glucagon receptor antagonist (GRA). Second, when high-concentration glucagon is administered together with glucose, GSIS is promoted through moderate activation of not only GcgR, but also GLP-1R in  $\beta$ -cells. This phenomenon is blocked by GLP-1R antagonist, Ex9-39, but not by GRA. Third, when a high concentration of GLP-1 is administered together with glucose, GSIS is potentiated through the complete activation of GLP-1R in  $\beta$ -cells, which is blocked by Ex9-39 (Figure 1).

First, they reported that first-phase and second-phase insulin secretion is even enhanced in a GLP-1 concentration-dependent manner under 16.7 mmol/L glucose in rat islets. Next, they then showed that GLP-1 not only increased GSIS in the first-phase from cAMP production, but also participated in cAMP-dependent insulin exocytosis in the second phase. The production of cAMP by GLP-1 was abolished by the cAMP inhibitor, Rp-8-Br-cAMP-pAB, and as a result, it was proved that it did not lead to the enhancement of GSIS in either phase. Furthermore, they evaluated the characteristic of glucagon-related insulin secretion in rat islets. Under the high-glucose condition, insulin secretion was enhanced in a glucagon concentration-dependent manner, with a threshold of 10 nmol/L in both first and second phases. This was a much higher concentration than the GSIS enhancement by GLP-1. In this condition, Rp-8-Br-cAMP-pAB even inhibited GSIS by glucagon stimulation. In contrast, low or intermediate concentrations of glucagon failed to enhance GSIS. As the reason, they supposed that endogenous glucagon binds to  $\beta$ -cell glucagon receptors to some extent from the beginning; therefore, a relatively lower concentration of glucagon is not enough to potentiate GSIS. In fact, they confirmed that glucagon is present in high concentrations in the condition medium of rat insulinoma cell line (INS-1), whereas GLP-1 is extremely low. In the next study, they evaluated the cAMP production under physiological conditions by a technique

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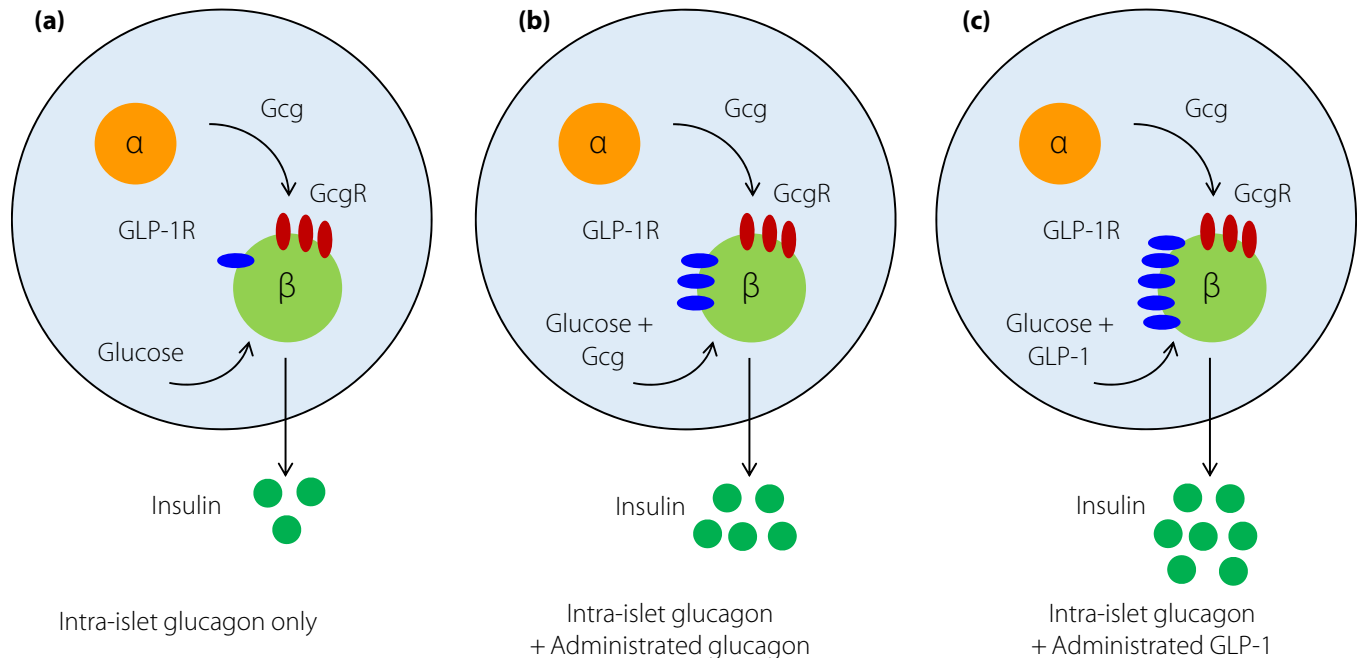
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**Figure 1** | Schema of glucose stimulated insulin secretion (GSIS) in response to various stimulations. Glucose (a) alone, (b) together with glucagon and (c) with glucagon-like peptide-1 (GLP-1). (a) Intra-islet glucagon occupies a large number of glucagon receptors (GcgR), but fewer GLP-1R. The intra-islet glucagon enables islets to show first-phase GSIS in response to glucose alone. (b) When a high concentration of glucagon is given in combination with glucose, resultant partial occupancy of the GLP-1R leads to potentiation of first-phase GSIS. (c) When high concentration of GLP-1 is given together with glucose, full GLP-1R are occupied, thereby potentiating first-phase GSIS. The original of this figure was shown in the previous report<sup>5</sup>.

called fluorescence resonance energy transfer (FRET) assay using human embryonic kidney cells 293 (HEK293) cells expressing GcgR and GLP-1R. Condition medium from INS1 cells did not promote the production of cAMP in HEK293 expressing GcgR, but promotes the production of cAMP in HEK293 having GLP-1R. These results strongly suggested that glucagon is also involved in the production of cAMP via the GLP-1 receptor of HEK293. These results based on fluorescence resonance energy transfer assay using HEK293 cells clearly suggested action sites of glucagon and GLP-1 on  $\beta$ -cells to enhance cAMP production. Specifically, GLP-1 acts at GLP-1R, and glucagon at GcgR or GLP-1R in a concentration-dependent manner.

Next, the authors investigated which phase is involved in the enhancement of GSIS by glucagon using a GRA. As a result, GRA inhibits first-phase GSIS in response to glucose alone, but is

ineffective when glucose is paired with glucagon. When 10 nmol/L glucagon is administered to the HEK293 cells having GLP-1 receptors, a reaction is observed regardless of the antagonism of the glucagon receptor. From this experiment, endogenous glucagon has a GLP-1R-mediated effect on first-phase GSIS enhancement, while administered glucagon strongly binds to the GLP-1R, enhancing GSIS in both phases. They then used the Ex9-39, and observed its effect on GSIS in rat islets and HEK293 cells. In conclusion, Ex9-39 inhibits first-phase GSIS in response to glucose alone, while also blocking the potentiation of GSIS by glucagon. Combined with GRA experiments, they show that endogenous glucagon has a first-phase GSIS-enhancing effect mediated by GLP-1 and glucagon receptors, and it is shown that the extrinsic high-concentration glucagon has a GSIS-enhancing effect mediated by the GLP-1R.

Although GRA and Ex9-39 antagonist action is readily studied in HEK293-H188 C24 cells that express the recombinant GcgR and GLP-1R, a more physiological approach is to evaluate antagonist action in INS-1832/13 cells that co-express endogenous GcgR and GLP-1R. That is, in INS1 cells, GRA suppressed the cAMP production in a concentration-dependent manner, but could not completely block it, whereas Ex9-39 could not suppress the production of cAMP at all. However, the addition of both antagonists completely suppressed the cAMP production. Finally, a dual agonist action of glucagon was established by showing additive actions of the GRA and Ex9-39 to suppress the cAMP production.

Generally, *in vitro* GSIS suddenly changes the environment of islets from a 2.8-mmol/L glucose solution to a 16.7-mmol/L glucose solution. The authors considered this a problem, and attempted to mimic the human oral glucose tolerance

test environment by gradually increasing the glucose concentration. Unlike its inhibitory effect in stepwise assays of first-phase GSIS, the GRA failed to alter insulin secretion stimulated by a glucose gradient alone. Similarly, Ex9-39 also failed to alter insulin secretion in response to a glucose gradient alone. Thus, GSIS stimulated by a glucose gradient is not conditional on intra-islet glucagon acting at the GcgR or GLP-1R. Still, glucagon (10 nmol/L) potentiated GSIS in the gradient assay, an action insensitive to the GRA, but blocked by Ex9-39. Furthermore, the GRA failed to block the potentiation of insulin secretion by GLP-1 (1 nmol/L), whereas Ex9-39 was effective. Thus, glucagon and GLP-1 exerted their stimulatory effects solely through the GLP-1R.

The findings reported by Cabrera *et al.*<sup>5</sup> provide a theoretical rationale to support a possibility that dual agonist peptides, which simultaneously stimulate the GLP-1R and GcgR, functionally restore the deranged GSIS in the diabetic condition. Based on this rationale, the mechanistic schema of GSIS in response to various stimulations, such as glucose alone, exogenous glucagon and exogenous GLP-1, is shown in Figure 1. The GcgR/GLP-1R co-agonist that stimulates both peptide signals is a clinically relevant tool as an antidiabetic drug, and is expected to be formulated in the future.

There were some limitations to the present study. The authors did not investigate the effects on GSIS by co-administering



GLP-1 and glucagon. Considering that dual agonists will appear in the future, it is important to use both at various concentrations and examine their effects on insulin secretion. Also of interest to readers is the effect of glucagon on non- $\beta$ -cells. The main actions of glucagon in the liver are glycogenolysis and gluconeogenesis. The effects of glucagon at concentrations that increase GSIS on  $\beta$ -cells on hepatocytes must be investigated in the future. GcgR are also expressed in the heart, gastrointestinal tract, kidneys, brain and adipocytes, and it is necessary to investigate the effects of glucagon on these organs at various concentrations.

Taken together, GLP-1R stimulation with GLP-1 not only enhances first-phase GSIS, but also leads to the recruitment of cAMP-dependent second-phase GSIS, which is absent in the absence of GLP-1. High concentrations of glucagon combine with GLP-1R to achieve the same effect as GLP-1. Glucagon acting on GcgR and GLP-1R has been shown to play an important role in systemic glucose homeostasis.

#### DISCLOSURE

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